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DIFFERENTIATION OF THE EFFECTS OF EXOGENOUS GROWTH HORMONE AND ENHANCED GROWTH RATE ON THE INCIDENCE OF OSTEOCHONDROSIS IN PIGS

by

PING HE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE
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F Aherne

.....

Dr. F.X. Aherne (Supervisor)

Signed by

.....

Dr. T. Basu

Al Schaefer

.....

Dr. A.L. Schaefer

J R Thompson

.....

Dr. J.R. Thompson

Robert Blair

.....

Dr. R. Blair (External examiner)

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ABSTRACT

Ractopamine supplementation of pig diets at 20 ppm enhanced growth rate and carcass leanness but had no significant effect on the incidence or severity of the joint cartilage damage in the humeral and femoral condyles. Uronic acid concentration in the cartilage was not affected by ractopamine supplementation.

Daily injection of pigs with recombinant porcine somatotropin (rpST) significantly increased average daily gain and carcass lean content compared to the control or rpST-slow-release implanted pigs. However, rpST treatment either by daily injection or slow release implant increased cartilage damage and decreased uronic acid concentration of the cartilage from the weight-bearing areas of both humeral and femoral condyles compared with the control pigs. These data suggest that the high incidence of OC observed with rpST treated pigs was related more to the rpST treatment per se than the mechanical stress to the joint from enhanced growth.

These results were confirmed in the third experiment in which daily injection of rpST did not increase growth rate compared with control pigs but did significantly increased the incidence of OC. Both uronic acid and hydroxyproline concentrations of the cartilage from the weight-bearing areas of humeral and femoral condyles were significantly reduced by rpST treatment of pigs from 33.0 to 100.3 kg live weight.

In the final experiment, the daily injection of rpST from 56.1 to 106.5 kg live weight again increased the incidence of OC lesions in joint cartilage. Histological examination of cartilage from the weight-bearing areas of humeral condyles showed OC lesions in the hypertrophic zone of the cartilage in rpST treated pigs. Injection of pigs with rpST reduced the concentrations of sulfated glycosaminoglycans, uronic acid and hydroxyproline in the middle and bottom layers of the joint cartilage compared with the control pigs. These data suggest that subarticular cartilage of rpST treated pigs is a biomechanically weak tissue and the reduction of concentrations of proteoglycan and collagen in the middle and bottom layers of cartilage may be through an inhibitory effect of somatostatin on chondrocyte secretion of insulin-like growth factor-I.

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I. GENERAL INTRODUCTION

The term osteochondrosis (OC) was first used by the Swedish scientists Ljunggren and Reiland in 1970 to describe a syndrome or a group of syndromes causing degenerative joint disease or limb deformities in pigs. A more exhaustive description of the disease was later presented by Grondalen (1974a). However, it has also been suggested that dyschondroplasia is a more accurate descriptive term than OC for the pathologic changes which primarily affect the growth plate and joint cartilage (Olsson 1978). Because of the widespread acceptance of the term OC, it was is used in this thesis. We define OC as a primary, noninflammatory disturbance in the joint cartilage and growth plate that results in failure of endochondral ossification.

Although growth performance of pigs up to market weight may not be severely affected by OC (Nakano et al. 1984; Brennan and Aherne 1986), it is an major cause of culling in the breeding herd (Friendship et al. 1986). Hill (1990) reported that breeding pigs culled because of lameness had a 100% frequency of OC or related cartilage damage. The financial loss due to OC in the United States alone in 1988-1989 was estimated at more than 24 million dollars (Hill, 1990). Therefore, OC is not only a animal welfare issue, but also an economic concern in swine production.

A. Histology and chemistry of joint cartilage

Joint cartilage is a connective tissue that is located

at the surface of diarthrodial joints. It acts as a cushion to sustain mechanical stresses, such as weight loading, wearing and friction, during daily activities of animals (Mow et al. 1992). There is another type of cartilage, namely physis located between the epiphysis and metaphysis. The physis is responsible for bone elongation during animal growth, but disappears around the time when the animal reaches puberty. Because of its location, the physis may be less important as a weight-bearing tissue than the joint cartilage (Nakano and Aherne, in press). Therefore, the present study focused on joint cartilage, even though OC develops in both cartilage tissues.

Light and low-power electron microscopy studies have shown that joint cartilage can be regarded as superficial, middle and bottom layers (Mow et al. 1992). The cells, namely chondrocytes, are morphologically different in these layers. The superficial layer consists of articular cartilage and prechondrocytes that undergo differentiation and proliferation during the process of bone growth (Kember 1983). Subsequently, the cells become mature in the middle layer, and then hypertrophy and are incorporated into epiphysis bone in the bottom layer (Mow et al. 1992). During these processes, chondrocytes are also responsible for the growth and maintenance of their surrounding matrix, including the turnover of the matrix molecules and preparation of cartilage for calcification.

Extracellular matrix of joint cartilage is composed of structural macromolecules, water and electrolytes (Poole 1986). Collagen is the major constituent of the macromolecules, comprising 50 to 80% of the dry weight or about 15 to 22% of the wet weight of the catilage (Mow et al. 1992). The predominant type of collagen in cartilage is type II with trace of other collagen types, such as types IX and XI (Kuettner et al. 1986). Each type II collagen fibre is made of three α1[II] polypeptide chains with repeating glycine-proline-X units with hydroxyproline being primarily incorporated in the X position (Kuettner et al. 1986). Collagen fibres form intermolecular crosslinks, such as dehydrohydroxylysinohydroxynorleucine and pyridinoline, to maintain the cohesiveness of the collagen network and provide high tensile strength to cartilage (Schmidt et al. 1990).

Proteoglycans comprise the second largest component of the macromolecules in the extracellular matrix of cartilage. These macromolecules contain protein cores to which are attached a number of covalently bound glycosaminoglycans (Poole 1986). About 90% of the glycosaminoglycans in cartilage is chondroitin sulfate and 8% keratan sulfate. Chondroitin sulfate is composed of repeating disaccharide units of uronic acid and sulafted N-acetylgalactosamine while keratan sulfate is composed of repeating disaccharide units of galactose and sulfated N-acetylglucosamine (Poole

1986). Aggregates are formed when many proteoglycan monomers bind to a long monofilament chain of hyaluronic acid and these aggregates are stabilized by a globular link protein (Zhu et al. 1991). The sulfate (SO_4) and carboxyl (COOH) groups on the glycosaminoglycan chains become ionized in cartilage. The distance between glycosaminoglycan chains are so close that the charged groups exert a strong charge-charge repulsive force on each other, which contributes up to 50% of the overall compressive strength of cartilage (Lai et al. 1989). In addition, proteoglycans form a network that regulates pore size and hydraulic permeability and makes cartilage stiffer and more viscous (Pottenger et al. 1982).

The maintenance of extracellular matrix is the responsibility of chondrocytes. There are some data in the literature which suggests that chondrocytes from different layers of cartilage have different metabolic and synthetic activities (Kuettner et al. 1986), so that the content of collagen and proteoglycan differ between layers (Ekman et al. 1990).

B. Growth rate and osteochondrosis

The etiology of OC in pigs is still unknown. Among the numerous factors suspected to contribute to the occurrence of OC, rapid growth and increased lean growth have been suggested to be of primary importance (Thurley 1965; Grondalen 1974b; Strittmatter et al. 1977; Lundeheim, 1987; Carlson et al. 1988a). The theory behind this suggestion is

that rapid growth rate and increased muscle mass may result in considerable mechanical stress on relatively immature joint cartilage and physis. This statement may attract much more attention from the swine industry in the future because pigs nowadays are growing faster and leaner than ever before.

Evidence to support the hypothesis that rapid growth rate increases the incidence of OC have been presented by several investigators. Grondalen and Vangen (1974) studied genetically selected fast and slow growing pigs and reported a higher incidence and severity of OC in the former. In agreement with their observation, Geodegebuure et al. (1980) also found that a fast-growing breed had more severe lesion score in joint cartilage and growth plates than a slow-growing breed of pigs.

In order to further study the relationship between growth rate and the occurrence of OC, researchers have reduced growth rate by restricted feeding (Fell et al. 1970; Grondalen 1974b; Nakano et al. 1979a; Arther et al. 1983) or by providing low nutrient density diets (Nakano et al. 1984; Woodard et al. 1987). However, none of these approaches significantly reduced the incidence or severity of OC, although growth rate was decreased to various degrees. Hanssen and Grondalen (1979) summarized six experimental results and found that restriction of energy intake of pigs from 25 to 100 kg live weight did not significantly affect

cartilage lesion score for the elbow and knee joints. Correlation coefficients were not significant between growth rate and cartilage lesion score in pigs with normal variation in growth rate (Nakano et al. 1984). Conversely, Reiland (1978) reported that slower growth rate achieved by restricted feeding resulted in less severe lesions in joint cartilage or growth plates. Unfortunately, the number of experimental animals used by Reiland (1978) was very small.

Pigs were slaughtered and cartilage damage was evaluated at a constant body weight in the aforementioned experiments, and therefore slower growing pigs had a longer time to develop OC than fast growing pigs, which may have confounded time on experiment and growth rate as causative factor in OC. Carlson et al. (1988b) fed two groups of pigs at 70% of the amount fed to a control group. Pigs restrict-fed had 30% lower growth rate compared with the control pigs. One group of slow growing pigs was slaughtered at the same age (208 days), while another group of slow growing pigs were slaughtered at the same body weight (110 kg), as the control pigs. Pigs with different growth rates that were slaughtered at the same age had the same incidence and severity of OC, while slow growing pigs slaughtered at the same weight as the control pigs had lower incidence and severity of OC in the humeral and femoral condyles as fast growing pigs. Their observation suggested that a cartilage repairing process must dominate during the extended growth

period for the later slaughtered slow-growing pigs. However, no evidence is available to support this speculation. Therefore, the observation reported by Carlson et al. (1988b) may not much help to convince readers that fast growth rate can be considered a primary etiological factor of OC.

C. Growth hormone, insulin-like growth factor-I and osteochondrosis

Among the numerous hormones and growth factors that influence cartilage and bone growth, growth hormone (GH) and insulin-like growth factor-I (IGF-I) are considered the most important. These hormones directly stimulate chondrocyte proliferation (Cheek and Hill 1974). In the superficial layer, GH binds to its receptor on the prechondrocytes and stimulates cell differentiation (Barnard et al. 1988). During the process of differentiation, cells become responsive to IGF-I, and the gene encoding for IGF-I is expressed, which results in an increased synthesis of IGF-I by chondrocytes (Isaksson et al. 1987). The locally produced IGF-I, which is the major source for growth plates, interacts with the receptors on the proliferating chondrocytes and promotes chondrocyte maturation and hypertrophy in the middle and bottom layers of the joint cartilage, respectively (Isaksson et al. 1987). As a result, the cartilage becomes thicker and longitudinal bones elongate.

However, evidence have been provided by many researchers that high plasma levels of growth hormone causes a high incidence of OC in different animals. Injection of exogenous growth hormone increased cartilage thickness, particularly in the zone of proliferating chondrocytes, but decreased the shearing strength of cartilage growth plates in rats (Harris 1950). Dogs treated with growth hormone developed severe OC (Paatssama et al. 1975). Chickens with high growth hormone levels had a higher incidence and severity of tibia dyschondroplasia, a disease similar to OC in mammals, than chickens with low growth hormone levels (Vasilatos-Younken and Leach 1986).

The utilization of recombinant porcine somatotropin (rpST) in experiments with pigs has provided an opportunity to study the relationship between exogenous growth hormone and the occurrence of OC. Pigs injected with toxic levels (0.22 mg or 1.10 mg per kg body weight per day) of rpST developed severe joint cartilage damage (Machlin, 1972). Carlson et al. (1988a) concluded that lesions involving joint cartilage and growth plate were more severe in pigs treated with rpST than in control pigs. A possible effect of impurity in the rpST used can be eliminated because a similar increase in the incidence of OC was observed in pigs treated with either rpST or pituitary porcine growth hormone (Evock et al. 1988).

D. Development of hypotheses

Studies comparing the concentration and activity of growth hormone in pigs with different growth rates revealed that high plasma growth hormone levels were associated with fast growing pigs (Lund-Larsen and Bakke 1975; Althen and Gerrits 1976; Wangness et al. 1981; Hoffman et al. 1983; Stone et al; 1985; Bark et al. 1988). Pigs from a fast growing line in the same herd had a higher frequency of OC in the femorotibial joint than slow growing lines (Grondalen and Vangen 1974). Geodegebuure et al. (1980) also reported a high incidence of OC in genetically selected fast growing pigs. When growth hormone is given as an exogenous source, such as rpST, an increased incidence or severity of OC are also observed (Machlin, 1972; Carlson et al. 1988a; Evock et al. 1988). These results suggest that it may not be the mechanical stress from the enhanced growth rate but the high level of plasma growth hormone that is the primary etiological factor associated with high incidence of OC in fast growing pigs.

However, there is still no information available regarding the independent effects of growth hormone and fast growth rate on the occurrence of OC in growing and finishing pigs. The effects of growth hormone per se on the incidence of OC is confounded with the mechanical stress due to enhanced growth in all experiments reported to date. Based on the above literature review, it is reasonable to

hypothesize that:

1). Increased growth rate or carcass lean content without a change in growth hormone levels in growing-finishing pigs may not cause a high incidence or severity of OC.

2). Increased growth hormone level without enhanced growth rate may affect the integrity of joint cartilage.

The present study was undertaken to examine these hypotheses by using growth promoters, such as the ß-adrenergic agonist ractopamine and rpST, to enhance growth rate or increase plasma growth hormone levels and to evaluate their effects on cartilage soundness in growing-finishing pigs.

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II. EFFECT OF RACTOPAMINE ON CARCASS CHARACTERISTICS AND JOINT-CARTILAGE SOUNDNESS IN FINISHING PIGS[1]

A. INTRODUCTION

Osteochondrosis (OC) is the focal failure of endochondral ossification and is one of the most common causes of lameness in swine (Reiland 1978). The pathogenesis of OC is not clear. A loss of proteoglycan in the joint cartilage, which results in a loss of compressive strength, is associated with the incidence of osteochondrosis (Nakano et al. 1987). Proteoglycan content of cartilage can be estimated from uronic acid analysis (Nakano et al. 1979a, b). Fast growth, particularly a high rate of lean tissue deposition, is suggested to be a major contributing factor (Kincaid and Lidvall 1983; Lundeheim 1987). However, a consistent association between growth rate and the incidence or severity of joint lesions has not been demonstrated. Nakano et al. (1984) reported that the growth rate of pigs fed adequate diets was not significantly correlated with the incidence and severity of joint lesions. Also, lowering growth rate by restricting feed intake did not significantly decrease the incidence and severity of osteochondrosis in pigs (Nakano et al. 1984; Woodard et al. 1987). Moreover,

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there is a lack of information on the effects of fast growth rate caused by growth promoters, such as ractopamine, on joint cartilage soundness.

Ractopamine hydrochloride is a synthetic ß-adrenergic agonist which mimics the activity of naturally occurring catecholamine through its interaction with ß-receptors on target tissues. It has been reported that ractopamine supplementation to pig diets increased body lean and decreased body fat content (Crenshaw et al. 1987; Nelson et al. 1987; Anderson et al. 1988; Watkins et al. 1990 and Stites et al. 1991). Feeding the ß-adrenergic agonist, cimaterol, to pigs increased the growth rate and the incidence of foot lesions (Jones et al. 1985), but had no significant effect on joint cartilage (Hill and Dalrymple 1987).

The objective of this study was to evaluate the effects of feeding two dietary levels of ractopamine on carcass characteristics, and on the incidence and severity of osteochondrosis in finishing pigs.

The present experiment was reviewed and approved by the Animal Care Committee, Agriculture Canada Research Station in Lacombe. The animals used in the present experiment were cared for under the guidelines of the Canadian Council on Animal Care.

B. MATERIALS AND METHODS

Initially, one hundred and fifty PIC pigs (Pig Improvement Company, Camborough X Canabrid, equal numbers of barrows and gilts) at an average live weight of 64.2±2.4 kg were selected for this experiment. Six pigs were slaughtered and dissected initially to serve as a baseline for estimating tissue deposition rates. One hundred and forty four pigs were blocked by sex and randomly assigned to fully slatted, 1.52 X 1.98 m pens. Each pen housed three pigs of the same sex and was treated as the experimental unit. The room temperature was maintained at 20°C with a 12 h light-dark cycle. The four experimental diets (Table 1) were formulated to contain 17 or 20% crude protein (with 0.8 or 1.1 % lysine, respectively) and 0 or 20 ppm ractopamine (Eli Lilly, Canada). Pigs were fed ad libitum and had free access to water. Feed consumption per pen and individual pig weights were recorded weekly.

All pigs were slaughtered at an average live weight of 102.4±2.7 kg in the Meat Research Centre, Agriculture Canada Lacombe Station. Forty eight pigs, one from each pen, were randomly selected for carcass evaluation. Fat depth and lean depth were measured at the 3rd and 4th rib, 70 mm from the carcass mid-line by a Hennessy Probe (Hennessy and Chong Ltd., New Zealand), and these measurements were used to predict carcass lean content and Hennessy index of the carcass (Anonymous 1986). The left half of the carcass was

chilled at 2°C for 2 d after slaughter and then stored at -18°C until dissection.

Carcass dissection was performed at the Agriculture Canada Research Station, Lacombe, Alberta. The left side of each carcass was first processed into primal cuts and then separated into fat, lean and bone following the procedures of Martin et al. (1981) and these values were used to estimate the dissection index of carcass (Anonymous 1986).

The humerus and femur, with intact elbow and knee joint from the left side of each carcass, were collected and stored frozen for subsequent cartilage evaluation. Cartilage soundness was scored following the system introduced by Brennan and Aherne (1986). About 2 g of cartilage from the weight bearing areas of the humeral and femoral condyles were taken for measurement of uronic acid concentration (Nakano et al., 1979a).

Total nitrogen content of the diets was determined by the Kjeldahl procedure (AOAC 1984) and crude protein concentration was calculated by multiplying nutrogen concentration by 6.25. Lysine level was determined by HPLC (Jones and Gilligan 1983) and dietary ractopamine concentration was analyzed by Eli Lilly Canada Inc., Calgary, Alberta, Canada.

Pig performance data were calculated based on total feed intake, total gain and total trial days. General Linear Model (GLM) procedures of SAS (SAS, 1985) were used for

analysis of effects of sex, protein and ractopamine (2 X 2 X 2) and their interactions. Primary analysis showed no significant interactions, therefore only the main effects are presented and discussed in the present report. All percentage data were normalized to avoid skew distribution. Initial weights of pigs were used for covariance analysis of sex differences in performance and carcass weights were used for covariance analysis of ractopamine effect on carcass characteristics. Pearson correlation coefficient procedures of SAS were used to evaluate the relationships between parameters.

C. RESULTS AND DISCUSSION

Animal performance

Twelve pigs were removed from the trial due to pneumonia (7), rectal prolapse (3), and extremely poor condition (2). Total feed intake and weight gain per pen were recorded at the time of moving the pigs. The data of these pigs were included in the analyses.

The effects of ractopamine, dietary protein and sex on pig performance are presented in Table 2. Compared with the control group, pigs fed the ractopamine supplemented diet had significantly increased daily gain ($P<0.005$) and decreased feed to gain ratio ($P<0.001$), with no significant difference in daily feed intake. Several researchers have demonstrated that daily weight gain and feed efficiency are

improved by feeding ractopamine, while the effect on feed intake is variable depending on the level of ractopamine in diet (see review by Stahly 1990). Aalhus et al. (1990) did not observe any significant improvement in pig performance when ractopamine was included in the diets at various levels (0, 10, 15 and 20 ppm). Bark et al. (1989) suggested that the response to ractopamine was greater with slower growing pigs than with faster growing pigs.

Dietary protein levels (17% plus 0.8% lysine vs 20% plus 1.1% lysine) did not influence pig performance significantly (Table 2). Previous studies have shown that ractopamine supplementation of diets containing 12% protein resulted in a 6% depression in the growth rate of finishing pigs compared with pigs fed the same diet without ractopamine (Anderson et al. 1987). Adeola et al. (1990) also reported a 12% decrease in daily gain of finishing pigs fed a 13% crude protein diet containing 20 ppm ractopamine. However, daily gain was enhanced by 7 to 11% in finishing pigs fed diets with 15 or 16% crude protein and supplemented with ractopamine (Crenshaw et al. 1987; Nelson et al. 1987; Anderson et al. 1988; Watkins et al. 1990 and Stites et al. 1991). Jones et al. (1988) reported no significant difference in the growth rate of pigs fed ractopamine supplemented diets containing 16, 20 or 24% crude protein. However, Dunshea (1991) estimated that a dietary protein content of 16.8% was at least required to support maximal

protein deposition rate for gilts receiving 20 ppm ractopamine. Thus, the response of ractopamine treated pigs to dietary protein level will depend on adequate amount, but not the levels above 17%, of dietary protein.

Effects of ß-adrenergic agonists are often tested with gilts and barrows and differences in performance due to sex in these experiments are frequently not significant (see Dunshea, 1991). However, Dunshea (1991) compared the effect of ractopamine on entire males, castrates and gilts and reported that the improvement in average daily gain was greatest for castrates ($P<0.05$) and least for entire males. He suggested that ractopamine treatment of growing pigs tended to equalize normally observed sex differences in performance. In the present experiment gilts had a lower feed intake, and feed conversion with similar average daily gain (Table 2) when compared to barrows, but the interaction between sexes and ractopamine was not significant. Therefore, the differences between barrows and gilts (Table 2) were considered to be due to the effects of sex, and not to ractopamine.

Carcass characteristics

Ractopamine induced changes in carcass characteristics, as measured by dissection and the Hennessy probe (Table 3). Carcass dissection showed that percentage dissectible lean was increased ($P<0.001$), and percentage dissectible fat was decreased ($P<0.001$), in carcasses from ractopamine treated

pigs. The reduction of body fat was mainly due to reduced subcutaneous fat ($P<0.001$). Percentage bone was also reduced ($P<0.05$) by feeding the ractopamine supplemented diet. The dissection index (Anonymous, 1986) was increased by approximately 7 points for pigs fed ractopamine ($P<0.001$). The Hennessy probe measurements indicated that ractopamine increased lean depth ($P<0.05$), but had no effect on carcass fat depth, predicted lean content or Hennessy index (Anonymous, 1988). Aalhus et al. (1990) also observed an inconsistency between Hennessy probe measurements and dissection data, suggesting that the Hennessy Grading Probe may not be sufficiently sensitive to measure the improvement in carcass composition induced by ractopamine.

Dietary protein levels had no significant effect on carcass composition (Table 3). The differences between gilts and barrows in carcass characteristics (Table 3) were as expected, and were not significantly affected by dietary ractopamine supplementation.

Tissue accretion rates

The average live weight of the six pigs slaughtered at the beginning of the experiment was 63.4±2.4 kg. There was no significant difference in the carcass composition between gilts and barrows, therefore, the data were pooled and used to estimate the tissue accretion rates of the 48 pigs slaughtered at market weight. The average weights of lean, fat and bone of the six pigs were 20.4, 13.6 and 6.2 kg,

respectively. Tissue accretion rates were calculated as: dissectible tissue weights of individual pigs slaughtered at market weight minus average dissectible tissue weight of pigs slaughtered at the start of the experiment, divided by the number of days the pigs were on test. The results are presented in Table 4.

Lean tissue accretion rate was more than 100 $g.d^{-1}$ greater in ractopamine fed pigs than in control pigs ($P<0.001$). Although fat and bone accretion rates appeared to be lower in ractopamine treated pigs, there were no significant differences between treatments. The difference in total tissue accretion rate between ractopamine treated and control pigs was 78.3 $g.d^{-1}$ (616.3 $g.d^{-1}$, -538.0 $g.d^{-1}$, Table 4), indicating that the 90 g/d improvement in average daily gain of the ractopamine treated pigs (Table 2) was due primarily to increased lean tissue growth.

Tissue accretion rates were not significantly ($P>0.05$) affected by dietary protein levels (Table 4), supporting the suggestion that 17% crude protein may fulfil the optimal requirements for growth of pigs fed 20 ppm dietary ractopamine on an ad libitum basis (Dunshea, 1991). Barrows had a higher rate of fat accretion ($P<0.001$) than gilts, but accretion rates for lean, bone or total tissue were not significantly different between gilts and barrows (Table 4).

Cartilage soundness and uronic acid concentration

Though ractopamine supplementation of pig diets enhanced growth rate and carcass leanness, it had no effect on the incidence or severity of joint cartilage damage (Table 5). It has also been reported that the ß-adrenergic agonist cimaterol had no significant effect on the incidence of foot lesions or osteochondrosis in pigs, although muscle content and muscle to bone ratio, but not the daily weight gain, were increased (Hill and Dalrymple, 1987). Several other researchers have also shown no significant relationship between growth rate and the incidence and severity of osteochondrosis (Nakano et al. 1979b; Arther et al. 1983; Nakano et al. 1984; Woodard et al. 1987). These data do not support the suggestion of Kincaid and Lidvall (1983) that increased lean growth rate increases the level of joint cartilage damage in pigs.

Uronic acid concentrations in cartilage were not affected by ractopamine supplementation (Table 5), and provided supporting data that ractopamine supplementation to pig diets has no detrimental effect on joint cartilage composition.

Correlations between visual scores and uronic acid concentrations of cartilage from each condyle with performance parameters and carcass characteristics are presented in Table 6. None of the correlations determined were significant, which, again, suggest that mechanical

stress due to enhanced growth rate or high lean growth rate may not cause an increased incidence or severity of cartilage damage. Our observations and the fact that degenerative cartilage lesions can be seen in pigs at 15 days of age (Hill et al. 1985) suggest that etiologic factors other than growth rate should be considered.

In conclusion, ractopamine fed to pigs at 20 ppm improved pig performance and carcass quality. Dietary protein level (17% and 20%) or sex (gilts and barrows) did not influence the response to ractopamine. The enhanced growth rate or increased lean tissue accretion resulting from dietary supplementation with 20 ppm ractopamine did not increase the incidence or severity of osteochondrosis. These results suggest that growth rate may not be the primary etiologic factor in the onset of osteochondrosis in pigs.

Table II.1. Composition of experimental diets

Crude Protein (%)	17		20	
Ractopamine (ppm)	0	20	0	20
Ingredient (%)				
Wheat	19.8	19.8	25.5	25.5
Barley	59.0	59.0	43.5	43.5
Soybean meal (48% CP)	15.5	15.4	25.3	25.2
Tallow	2.0	2.0	2.0	2.0
Limestone	1.2	1.2	1.2	1.2
Dicalcium phosphate	1.0	1.0	1.0	1.0
Iodized salt	0.5	0.5	0.5	0.5
Vitamin premix[x]	1.0	1.0	1.0	1.0
Ractopamine premix[y]	-	0.1	-	0.1
Chemical composition (as fed basis)[z]				
Dry matter (%)	88.2	88.1	88.1	88.6
DE ($MJ.kg^{-1}$)	13.47	13.47	13.78	13.78
Crude protein (%, N x 6.25)	16.8	18.0	20.2	21.0
Lysine (%)	0.78	0.83	1.03	1.10
Ca (%)	0.75	0.75	0.77	0.77
P (%)	0.63	0.63	0.66	0.66
Ractopamine (ppm)	0	21.43	0	21.75

[x]Vitamin premix supplied the following per kg of diet: 6,600 IU vitamin A, 1,400 IU vitamin D, 40 IU vitamin E, 4 mg menadione sodium bisulfite, 8 mg riboflavin, 40 mg niacin, 20 mg d-calcium pantothenate, 40 µg vitamin B_{12} and 100 mg choline chloride.
[y]Ractopamine premix contained 2% ractopamine in soybean meal.
[z]Determined values of 6 samples per diet. Digestible energy, calcium and phosphorus were calculated.

Table II.2. Effects of ractopamine, dietary protein and sex on pig performance

	Ractopamine (ppm)			Protein (%)			Sex			
	0	20	Sig.[z]	17	20	Sig.[z]	Gilt	Barrow	Sig.[z]	SE[y]
Number of pens	24	24		24	24		24	24		
Initial wt. (kg)	64.08	64.17	NS	64.04	64.21	NS	62.75	65.50	*	0.87
Final wt. (kg)	101.95	102.73	NS	102.72	101.96	NS	102.45	102.23	NS	0.37
Feed intake.d^{-1} (kg)	2.75	2.78	NS	2.79	2.74	NS	2.65	2.88	***	0.05
Weight gain.d^{-1} (kg)	0.80	0.89	***	0.85	0.85	NS	0.85	0.85	NS	0.02
Feed/gain	3.44	3.14	****	3.31	3.27	NS	3.15	3.44	****	0.04

[z]Significance: NS, not significantly different (P>0.05); *=P<0.05; ***=P<0.005; ****=P<0.001.

[y]Standard error for means of ractopamine, protein and sex treatments.

Table II.3. Effects of ractopamine, dietary protein and sex on carcass characteristics[z]

	Ractopamine (ppm)			Protein (%)			Sex			
	0	20	Sig.[w]	17	20	Sig.[w]	Gilt	Barrow	Sig.[w]	SE[v]
Carcass weight (kg)[y]	64.37	66.17	*	65.35	65.19	NS	64.92	65.63	NS	0.56
Carcass characteristics[x]										
Total lean (%)	53.3	57.4	****	54.7	56.0	NS	57.1	53.6	****	0.58
Total fat (%)	36.0	32.4	****	34.9	33.4	NS	32.2	36.1	****	0.67
Subcutaneous (%)	21.1	18.7	****	20.3	19.4	NS	18.4	21.3	****	0.46
Intermuscular (%)	4.6	4.3	NS	4.6	4.4	NS	4.2	4.7	**	0.13
Total bone (%)	11.4	10.8	*	11.1	11.2	NS	11.3	10.9	NS	0.17
Dissection index	105.1	112.0	****	107.7	109.4	NS	111.2	105.9	***	1.09
Hennessy probe measurements										
Fat depth(mm)	16.0	15.3	NS	16.0	15.3	NS	14.5	16.7	**	0.58
Lean depth (mm)	44.7	49.3	*	45.8	48.2	NS	47.3	46.7	NS	1.32
Predicted lean (%)	51.2	51.8	NS	51.3	51.7	NS	51.9	51.1	**	0.23
Hennessy index	108.6	109.6	NS	108.5	109.7	NS	110.0	108.3	***	0.52

[z]The data was derived from 48 pigs.
[y]Cold carcass weight excluding head and feet.
[x]Percentage of carcass weight.
[w]Significance: NS=not significantly different (P>0.05); *=P<0.05; **=P<0.01, ***=P<0.005; ****=P<0.001.
[v]Standard error for means of ractopamine, protein and sex treatments.

Table II.4. Effects of ractopamine, dietary protein and sex on tissue accretion rate[z,y]

	Ractopamine (ppm)			Protein (%)			Sex			
	0	20	Sig.[x]	17	20	Sig.[x]	Gilt	Barrow	Sig.[x]	SE[w]
Lean accretion ($g.d^{-1}$)	302.1	409.0	****	344.8	366.3	NS	360.6	350.5	NS	18.3
Fat accretion ($g.d^{-1}$)	212.9	185.9	NS	209.4	189.4	NS	161.6	237.3	****	13.0
Bone accretion ($g.d^{-1}$)	23.0	21.4	NS	21.4	23.1	NS	22.6	23.9	NS	2.46
Total ($g.d^{-1}$)	538.0	616.3	*	575.3	578.9	NS	544.7	610.0	NS	24.8

[z]Tissue deposition rates were calculated as: (Dissectible tissue weight at the end of the trial - tissue weight of pigs slaughtered at the beginning)/days on trial.
[y]Data were obtained from 48 pigs.
[x]Significance: NS=not significantly different (P>0.05); *=P<0.05; ****=P<0.001.
[w]Standard error for means of ractopamine, protein and sex treatments.

Table II.5. Effects of ractopamine on joint cartilage soundness and uronic acid concentration[z]

	Ractopamine (ppm)			
	0	20	Sig.[y]	SE[x]
Average visual score[w]				
Humeral condyle	2.3	2.2	NS	0.18
Femoral condyle	2.1	2.2	NS	0.16
Percentage visual score of 3 and above				
Humeral condyle	29.2	33.3		
Femoral condyle	25.0	33.3		
Uronic acid (% dry matter)				
Humeral condyle	7.4	7.4	NS	0.29
Femoral condyle	7.7	8.0	NS	0.38

[z]Data were obtained from 48 pigs.
[y]Significance: NS=not significantly different (P>0.05).
[x]Standard error.
[w]See Materials and Methods.

Table II.6. Correlation coefficients between visual score, uronic acid concentration and carcass traits[z,y]

	Distal humeral condyle		Distal femoral condyle	
	Visual score	Uronic acid	Visual score	Uronic acid
Visual Score		0.010		-0.116
Uronic acid	0.010		-0.116	
Weight gain	-0.052	0.116	-0.304	-0.240
Lean accretion	-0.008	-0.087	0.028	-0.013
Bone accretion	0.195	-0.097	0.237	0.129

[z]Pearson correlation coefficients.
[y]Data were obtained from 48 pigs.

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III. DIFFERENTIATION OF THE EFFECTS OF GROWTH HORMONE AND ENHANCED GROWTH RATE ON THE OCCURRENCE OF OSTEOCHONDROSIS IN PIGS

A. INTRODUCTION

Osteochondrosis (OC) is a noninfectious syndrome manifested as failure of endochondral ossification in joint cartilage (articular cartilage-subarticular growth cartilage complex) and physis of long bones of growing animals. Because osteochondrotic lesions are consistently observed in the weight bearing area of the humeral and femoral condyles, mechanical stress is suggested to be an important etiological factor (Nakano et al. 1987; Brennan and Aherne 1986). Factors in addition to local overloading of joint cartilage that have been implicated in the incidence and severity of OC include hormonal disturbances. Hormonal influences on the integrity of the joint cartilage have been the subject of many studies. Growth hormone, either endogenous or exogenous, has been shown to contribute to several cartilage diseases, including OC in dogs (Paatsama et al. 1971), chickens (Vasilatos-Younken and Leach 1986) and pigs (Grondalen and Vangen 1974; Lund-Larsen and Bakke 1975; Etherton et al. 1986). Several investigators (Machlin 1972; Evock et al. 1988) reported that an increased

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incidence of OC has also been observed in pigs injected with recombinant porcine somatotropin (rpST). However, none of the aforementioned studies were able to separate the effects of hormonal disturbance from the local mechanical stress on joint cartilage resulting from the hormonally stimulated increased growth rate. Although the effect of fast growth rate on the incidence of OC has been studied for decades (Nakano et al. 1987), the relationship between growth rate and growth hormone in the occurrence of OC in pigs is unknown.

The objective of this experiment was to differentiate the effects of exogenous growth hormone and enhanced growth rate on the incidence and severity of OC in growing pigs.

The present experiment was reviewed and approved by the Animal Care Committee, Agriculture Canada Research Station in Lacombe. The animals used in the present experiment were cared for under the guidelines of the Canadian Council on Animal Care.

B. MATERIALS AND METHODS

One hundred and twenty Yorkshire pigs (60 gilts and 60 barrows) were selected between 65 and 75 kg body weight and randomly assigned on the basis of weight and sex to one of three treatments: control (sham implant), implant with prolonged release of rpST at 2 mg/day or a subcutaneous daily injection of 2 mg rpST into the dorsal neck region

near the base of the ear. Each treatment group was represented by ten pens of four pigs (five pens of gilts and five pens of barrows). Pens were approximately 2 m by 1.5 m in size. The barn temperature was controlled at 18°C. All animals were ad libitum fed a 17% crude protein, 1.18% lysine barley-wheat based diet which met or exceeded NRC (1988) recommendations for other nutrients (Table 1). Pigs had free access to water during the experimental period. A two week conditioning period to accustom pigs to the diet was provided. The pigs received their respective treatments from 70±5 kg body weight until 96±5 kg body weight. All animals were weighed weekly until slaughtered. Feed intake per pen was recorded on a weekly basis.

Upon reaching 95 kg, pigs were slaughtered and 60 pigs (two from each pen) were randomly selected for carcass evaluation. Pigs were transported (approximately 0.5 km) to the Meat Research Centre at the Lacombe Research Station, weighed, stunned by electrical shock, bled, scalded and dressed following normal commercial procedures. On the day after slaughter, the right side of each carcass was divided into primary cuts and dissected into fat, lean and bone. The humerus and femur with intact proximal and distal condyles from the right side of each carcass were collected at the time of dissection and kept frozen at -30°C until analysis.

Before analysis, the bones were thawed at room temperature. The cartilage soundness of the proximal and

distal humerus and femur was scored from 1 (normal) to 5 (severe damage), as described by Brennan and Aherne (1986). Uronic acid concentration, which indicates the concentration of glycosaminoglycan in the cartilage, was determined using cartilage from the weight bearing areas of humeral and femoral condyles (Nakano et al. 1979).

Analysis of the data was conducted using the General Linear model procedures of the Statistical Analysis System (SAS, 1982) with a model which included effects of sex, treatment and their interaction. The statistical differences between means at $P<0.05$ or greater were considered significantly different.

C. RESULTS

Pig performance data are presented in Table 2. Daily injection of pigs with rpST significantly increased the average daily weight gain ($P<0.0001$) compared with the control group. Daily weight gain was not significantly different between animals treated with rpST by means of the slow release implant and the control animals. Pigs injected with rpST had lower daily feed intake than the control group ($P<0.0001$) while pigs with the slow release implant had an intermediate daily feed intake. The feed/gain ratios were similar between the control and slow release implant groups but were lower for the daily injected animals ($P<0.0001$).

Carcass dissection data are presented in Table 3. Daily

injection of rpST increased carcass lean content ($P<0.0001$) and reduced carcass fat content ($P<0.0001$). The slow release rpST implant had no significant effect ($P>0.05$) on carcass characteristics compared with the control animals. The proportion of carcass bone was not affected ($P>0.05$) by either daily injection or slow release implant of rpST.

There were no differences in visual score or uronic acid concentration between sexes or proximal and distal condyles within the same treatment, therefore, the results were pooled to determine the treatment effect (Table 4). Porcine somatotropin given either by daily injection or by slow release implant increased the cartilage visual score of humeral condyles ($P<0.05$) but not of the femoral condyles. The percentage of humeral condyles with visual scores equal to or greater than 3, which indicated relatively severe cartilage damage, was also high in pigs treated with rpST. Uronic acid concentration in the cartilage from both humeral and femoral condyles was reduced in both groups of rpST treated pigs ($P<0.05$).

The relationship between the visual score, uronic acid concentration of joint cartilage and growth performance and carcass measurements are presented in Table 5. The correlation coefficient between cartilage visual score and uronic acid concentration was significant ($P<0.04$) for the femoral condyle but marginally not significant for the humeral condyle ($P<0.08$). Daily weight gain and carcass

characteristics were not significantly correlated with cartilage uronic acid concentrations of either condyles. However, a significant effect of rpST on cartilage uronic acid concentrations of both humeral ($P<0.03$) and femoral condyles ($P<0.01$) was found when the data were analyzed using the ANOVA procedures of SAS (Table 5).

D. DISCUSSION

Because a high incidence of OC has been occasionally observed in pigs with high growth rate, some investigators have speculated that excessive growth hormone may be a causative factor (Ljunggren and Reiland 1970; Grondalen and Vangen 1974; Lund-Larsen and Bakke 1975). This speculation was further supported by the observation that OC was more prevalent in pigs injected with rpST (Evock et al. 1988). Machlin (1972) described an arthritis-like damage to the joint cartilage of pigs injected with toxic levels (0.22 to 1.10 mg/kg live weight/day) of growth hormone. Evock et al. (1988) reported a 50% increase in the incidence of OC in pigs injected with rpST. Carlson et al. (1988) observed that cartilage growth plates were more severely damaged in pigs treated with rpST than in non-treated pigs. Although these experimental data strongly suggested that growth hormone has a detrimental effect on cartilage integrity, it was not clear if the increased mechanical stress on the cartilage due to the enhanced growth rate in rpST treated pigs also

played an etiological role in the occurrence of OC. The current experiment is the first to differentiate the rpST effect from the effect of increased growth rate which presumably resulted in a greater mechanical stress on the cartilage. Both daily injection and slow release implants of rpST induced a high incidence of osteochondrotic lesions in joint cartilage, although only the daily injection of rpST increased the growth rate and carcass lean content. These data demonstrated that rpST caused a high incidence of OC that was unrelated to growth rate and was not associated with an increased mechanical stress on the cartilage.

Although growth hormone has been known as a major stimulator of cartilage growth (Canalis 1983; Centrella and Canalis 1985; Isaksson et al. 1987), exogenous growth hormone, however, induces severe cartilage damage in some animals. Dogs injected with growth hormone developed severe OC (Paatsama et al. 1971). Chickens with a high incidence of tibial dyschondroplasia, a cartilage disease similar to OC in pigs, had higher secretory capacity and plasma circulating levels of growth hormone (Vasilatos-Younken and Leach 1986). The shearing strength of growth cartilage was reduced when gonadectomized rats were treated with anterior pituitary extract (Harris 1950). The present experiment demonstrates that uronic acid concentration is decreased in pigs treated with rpST. Over 90% of the glycosaminoglycans in the cartilage contain uronic acid. Glycosaminoglycans are

covalently attached to protein to form proteoglycans which aggregate with hyaluronic acid to provide cartilage its compressive strength (Poole, 1986). It may be speculated that rpST indirectly reduced cartilage compressive strength by decreasing the uronic acid concentration. Nakano and Aherne (1993) recently suggested that the subarticular growth cartilage, where early lesions occur, is a bio-mechanically weaker tissue than the articular cartilage and that excessive mechanical stress added to the subarticular growth cartilage impairs normal function of the extra cellular matrix and chondrocytes resulting in matrix destruction. Our data, however, suggest that growth hormone may also be involved independently of mechanical stress. This suggestion is consistent with that of Grondalen and Vangen (1974) and Lund-Larsen and Bakke (1975) that the high incidence of OC in pigs selected for high growth rate is due more to increased growth hormone activity than to increased mechanical stress on the joint.

The results from present experiment indicate that high level of plasma growth hormone is a more important factor than mechanical stress from enhanced growth rate in the occurrence of OC in pigs. It may explain why suppressed growth rate achieved by restricted feed intake (Fell et al. 1970; Grondalen, 1974, Nakano et al. 1979; Arther et al. 1983) or reduced dietary nutrient density (Nakano et al. 1984; Woodard et al. 1987) during the growing period did not

significantly reduce the incidence or severity of OC in pigs, because growth hormone levels may not be affected by energy intake (Campbell et al. 1988). Data from our previous experiment (Chapter II) further confirm the increase of growth rate through non-growth hormone axes, such as feeding ß-adrenergic agonist supplemented diet, had no influence on the integrity of joint cartilage in pigs.

Etiological studies on OC are difficult to conduct because of the unavailability of a suitable animal model. Kato and Onodera (1988) experimentally induced OC in rats by ofloxacin, a quinolone antibacterial agent. Animals treated with growth hormone may be a better model for the study of OC because the hormone directly regulates chondrocyte differentiation, maturation and indirectly controls the cartilage calcification process (Isaksson et al. 1987). More detailed information regarding the histological and biochemical changes in the cartilage of growth hormone treated animals is required.

Table III.1. Experimental diet composition

Ingredient (%)	
Barley	58.6
Wheat	20.0
Soybean meal (46.5% CP)	10.0
Meat meal (50% CP)	2.9
Canola meal (36% CP)	5.5
Dicalcium phosphate	0.8
Limestone	1.0
NaCl	0.5
Vitamin-mineral premix[z]	0.3
Lysine HCl	0.245
Choline chloride	0.055
Nutrient[y]	
Digestible energy (MJ/kg)	12.9
Crude protein (%, N x 6.25)	17.0
Calcium (%)	0.86
Phosphorus (%)	0.68
Lysine (%)	1.18

[z]Premix provided the following per kilogram of feed: vitamin A, 4,000 IU; vitamin D, 600 IU; vitamin E, 25 IU; potassium, 2.5 g; thiamine 1 mg; riboflavin 3 mg; niacin 21 mg; pyroxidine 1.1 mg; vitamin B_{12}, 20 ug; pantothenic acid, 17 mg; folic acid, 0.6 mg; biotin, 0.2 mg; iron, 150 mg; zinc, 150 mg; manganese, 30 mg; copper, 25; iodine, 0.2 mg; cobalt 0.4 mg and selenium 0.1 mg.
[y]As fed basis. Digestible energy was calculated.

Table III.2. Effect of rpST on pig performance

	Slow release	Daily injection	Control	SE[z]	Sig.[y]
Number of pens	10	10	10		
Number of pigs per pen	4	4	4		
Initial weight (kg)	69.2	68.6	68.9	1.01	NS
Final weight (kg)	102.8	103.7	101.4	0.93	NS
Daily weight gain (kg)	0.94[b]	1.10[a]	0.98[b]	0.02	****
Daily feed intake (kg)	2.58[ab]	2.54[b]	2.77[a]	0.06	****
Feed/gain	2.74[a]	2.31[b]	2.81[a]	0.05	****

[z]Standard error.
[y]Significant difference at ****=P<0.0001, or not significantly different (NS) at P>0.05.
[ab]Values with different superscripts within a row are significantly different.

Table III.3. Effect of rpST on pig carcass characteristics

	Slow release	Daily injection	Control	SE[z]	Sig.[y]
Number of animals	20	20	20		
Cold carcass weight (kg)	64.9	65.5	64.2	0.64	NS
Dissectible lean (g/kg)	603.5[b]	629.8[a]	604.9[b]	4.32	****
Dissectible fat (g/kg)	282.3[a]	258.0[b]	286.8[a]	4.79	****
Dissectible bone (g/kg)	114.1	112.2	108.4	2.08	NS

[z]Standard error.
[y]Significant difference at ****=P<0.0001, or not significantly different (NS) at P>0.05.
[ab]Values with different superscripts within a row are significantly different.

Table III.4. Effect of rpST on visual scores and uronic acid concentrations of joint cartilage from both proximal and distal humeral and femoral condyle of pigs

	Slow release	Daily injection	Control	SE[z]	Sig.[y]
Number of animals	20	20	20		
Visual score[2]					
Humeral condyle	3.3[a]	3.3[a]	2.0[b]	0.27	*
Femoral condyle	2.3	1.8	1.8	0.14	NS
Percentage of joints with visual scores equal to or greater than 3					
Humeral condyle	59.1	70.0	15.8		
Femoral condyle	17.4	18.2	10.5		
Uronic acid (% of dry matter)					
Humeral condyle	4.9[b]	5.4[ab]	6.4[a]	0.43	*
Femoral condyle	5.4[b]	5.3[b]	6.6[a]	0.39	*

[z]Standard error.
[y]Significant difference at *=P<0.05 or NS=not significant.
[2]1=normal and 5=severe damage.
[ab]Values with different superscripts within a row are significantly different.

Table III.5. Correlation coefficients between cartilage, growth performance and carcass measurements

	Uronic acid concentration	
	Humeral condyle	Femoral condyle
	Pearson correlation coefficient	
Average daily gain	0.072 (0.58)[z]	-0.083 (0.52)
Carcass lean content	0.042 (0.74)	-0.098 (0.45)
Carcass index	0.112 (0.39)	0.049 (0.71)
Visual score of Humeral condyle	-0.233 (0.08)	
Visual score of femoral condyle		0.268 (0.04)
	Index of correlation[y]	
Growth hormone treatment	0.278 (0.03)	0.334 (0.01)

[z]Correlation coefficient (probability).
[y]The index of correlation was the R value from the ANOVA Procedure of SAS.

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IV. EFFECTS OF RECOMBINANT PORCINE SOMATOTROPIN (rpST) ON JOINT CARTILAGE AND AXIAL BONES IN GROWING AND FINISHING PIGS

A. INTRODUCTION

It has been suggested that high levels of plasma growth hormone are associated with the occurrence of osteochondrosis (OC) in pigs (Carlson et al. 1988). Administration of recombinant porcine somatotropin (rpST) to pigs resulted in an increased incidence of OC (Machlin 1972; Grondalen and Vangen 1974; Lund-Larsen and Bakke 1975; Etherton et al. 1986; Evock et al. 1988). However, pigs injected with rpST usually have enhanced growth rate which has been considered an important predisposing factor to OC (Thurley, 1965; Grondalen 1974; Strittmatter et al. 1977; Lundeheim, 1987). Our previous experiment denonstrated that rpST treatment in pigs from 68.9 to 102.6 kg live weight resulted in a high incidence and great severity of OC, and this hormonal effect on cartilage was independent of the increased mechanical stress on joint cartilage due to increased growth rate (Chapter III). However, it is not known if rpST caused cartilage damage is associated with long term treatment, and if withdrawal of rpST injection before slaughter will reduce the incidence of OC in

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finishing pigs.

Studies of the effect of rpST on the physical properties of pig bones indicate that bone strength is decreased (Goodband et al. 1993). It have been reported that limb bone from rpST treated pigs have an increased water content (Caperna et al. 1989) and reduced ash content (Goodband et al. 1993). However, there is limited imformation regarding rpST effect on axial bones such as vertebrae or ribs.

The purpose of the present experiment was to evaluate the effect of rpST on the occurrence of OC and on the chemical composition of cartilage in growing and finishing pigs. The physical properties of vertebrae and ribs of pigs treated with rpST was also determined.

The experiment was approved by the University of Alberta Animal Care Committee to ensure adherence to Canadian Council of Animal Care guidelines.

B. MATERIALS AND METHODS

Seventy two gilts (PIC, Camborough) averaging 20 kg liveweight were allotted to four pens (6 m X 4.3 m) with partially slatted concrete floors. Each pen contained two electronic feeders (Hunday Electronic Ltd., Newcastle, UK) and pigs were fitted with electronic ear tag transponders. Pigs in two pens were choice-fed isoenergetic diets containing 24% crude protein (CP) or 10% CP in different

feeders. Pigs in the other two pens were provided with a single diet of 18% CP during the conditioning period (20 to 33 kg), 16% CP diet from 33 to 63 kg and 14% CP from 63 to 100 kg liveweight. Ingredient and nutrient composition of the diets are presented in Table 1. Pigs were fed ad libitum and had free access to water throughout the experiment.

Pigs were allowed a 20 d conditioning period to become accustomed to the feeding system. At the end of the conditioning period, two pigs from each pen were randomly selected and slaughtered for carcass dissection. The remaining pigs, with an average liveweight of 33.0±2.5 kg, were randomly assigned to one of three treatments: daily saline injection (control, eight pigs per pen, 32 pigs in total), daily injection of recombinant porcine somatotropin (rpST, Pitman-Moore, Terre Haute, IN) at 100 µg/kg liveweight (five pigs per pen, 20 pigs in total), and a similarly treated rpST group which was switched to saline injection at 62.8±3.7 kg liveweight (withdrawal, three pigs per pen, 12 pigs in total). Injections were performed daily from 0800 to 0900 h on alternative sides of the dorsal neck region near the base of the ear. The pigs were weighed weekly and the dosage of rpST or the amount of saline was adjusted based on individual liveweight.

Upon reaching approximately 62.8±3.7 kg live weight, eight pigs from each of the control and rpST treatment groups, two from each pen, were randomly selected for

slaughter. The remaining pigs were slaughtered at 100.3±5.5 kg. At slaughter, pigs were weighed and stunned by electric shock (400 Volt for 2 to 3 sec), bled, scalded and dressed following normal commercial procedures. The carcasses were chilled for 24 h at 1°C and the right side of each carcass was dissected into fat, lean and bone. The percentage of carcass tissue was calculated as (tissue weight/weight of half carcass) X 2 X 100, head and feet were excluded from the carcass. The rate of tissue accretion was calculated as (tissue weight at slaughter - average tissue weight of pigs slaughtered at 33.0 or 62.8 kg liveweight)/days on experiment. The distal humerus and proximal radius-ulna with intact elbow joint, the distal femur and proximal fibula-tibia with intact knee joint, the last thoracic vertebra and the last rib were collected at the time of dissection, sealed in plastic bags and stored at -30°C until analysis.

Blood samples were collected from pigs at 62.8 and 100.3 kg live weight. At each collection blood samples were taken at 3, 6 and 24 h after rpST or saline injection. Blood samples were taken from the jugular vein using a 14-gauge needle and heparin was used as anticoagulant. Plasma was separated from blood cells within 2 h of collection by centrifuging at 3500 x g for 15 min. Plasma growth hormone concentrations were determined using the homologous radioimmunoassay procedure of Marple and Aberle (1972) with modification: Antiporcine growth hormone (anti-GH-202-8)

D.N. Marple, Auburn University, AL) antisera and normal guinea pig serum (Calbiochem, La Jolla, CA) were used at final dilutions of 1:800000 and 1:4000, respectively. Purified porcine growth hormone (USDA-GH-I-2; USDA Animal Hormone Program, Beltsville, MD) was used as serially diluted standard (0.04-10 ng/tube) and USDA-GH-PPI (USDA Animal Hormone Program, Beltsville, MD) as radioiodinated antigen. The bound antigen was precipitated using a locally-raised horse anti-guinea pig gammaglobulin (final dilution 1:25) added in a 6% polyethylene glycol solution (PEG 8000; Fisher, Nepean, ON). Addition of 0.15, 0.3, 0.6, 1.2 and 2.4 ng of GH standard to 200 µL of control plasma and assay of 10, 25, 50, 100, 250 and 500 µL of the control plasma pool yielded quantitative recoveries of added hormone (103.3 SD 2.8%) and parallel inhibition curves. Sensitivity defined as 2.5 x SD of the zero standard, was 0.03 ng per tube. All measurements were performed in duplicate for each sample.

The last thoracic vertebra and last rib from the right side of each carcass were thawed at room temperature in a tightly sealed plastic bag to prevent moisture loss. After removal of all soft tissues bone weights were recorded. The volume of each bone was measured by immersing in water in a graduated cylinder. Bones were soaked in water for 5 min before placement in the graduated cylinder to prevent errors due to water absorption by bones. Constant dry weights of the bones were recorded after drying the bones in an oven at

110°C for 24 h. The bones were then ashed in an oven at 600°C for 24 h and ash weights were recorded.

Cartilage soundness of the distal humeral and femoral condyles and the growth plate of distal ulna were visually scored following the procedure described by Brennan and Aherne (1986). The width of physis in the proximal ulna was measured at three points along the vertical section of the epiphysis. To evaluate the effect of rpST on cartilage chemical composition, samples from the weight bearing area of humeral and femoral condyles were analyzed for concentrations of uronic acid and hydroxyproline following the procedures described by Nakano et al. (1979) and Stegemann and Stalder (1967), respectively.

Statistical analysis was performed using the General Linear Model procedures of the Statistical Analysis System (SAS, 1982). Initial liveweight was included in the model as a covariate for analysis of growth performance, carcass characteristics and tissue accretion data. To focus the discussion on the relationship between hormonal treatments and osteochondrosis in the different growth stages, feeding system was removed from the model after it was confirmed that there was no interactive effect of the feeding system with other factors on the parameters measured in the experiment. Each pig was an experimental unit. All percentage data were checked for normal distribution before further analysis.

C. RESULTS

Growth performance

During the growing period, daily injection of rpST decreased daily feed intake ($P<0.05$) and the feed/gain ratio ($P<0.05$) but did not affect daily weight gain compared to pigs in the control group (Table 2). During the finishing period, daily feed intake by pigs injected with rpST was lower than that by the control pigs ($P<0.05$), while daily weight gain and feed/gain ratio were not significantly different between these two groups. Feed intake of pigs withdrawn from rpST at 62.8 kg was greater than that of pigs that remained on the rpST treatment ($P<0.05$) while daily weight gain was lower ($P<0.05$) and feed/gain ratio was higher ($P<0.05$) than that of pigs on either control or rpST treatment (Table 2). Over the whole experimental period (33 to 100 kg) rpST injection at 100 µg/kg live weight reduced daily feed intake ($P<0.05$) and feed/gain ratio ($P<0.05$) but did not affect daily weight gain compared with pigs injected with saline. Withdrawal of rpST injection at 60 kg live weight resulted in a reduced daily weight gain ($P<0.05$) and increased daily feed intake ($P<0.05$) compared to pigs that remained on the rpST treatment, and these pigs had the highest ($P<0.05$) feed/gain ratio among the three groups of pigs (Table 2).

Carcass characteristics

Daily injection of rpST during the growing period

reduced ($P<0.05$) carcass fat and increased ($P<0.05$) carcass lean but did not increase percentage bone compared to saline injection (Table 3). Injection of rpST from 33.0 kg to 100.3 kg liveweight decreased ($P<0.05$) percentage fat in the carcass and increased ($P<0.05$) percentage lean and bone. The effect of rpST treatment during the growing period was negated by its withdrawal at 62.8 kg. At a slaughter weight of 100.3 kg liveweight, the withdrawal group had similar carcass fat, bone and lean percentages as the control group (Table 3).

Tissue accretion rate

Lean tissue accretion was increased ($P<0.01$) in the finishing and overall experimental periods while fat accretion was decreased ($P<0.01$) in both experimental periods by rpST injection compared to saline injection (Table 4). Withdrawal of rpST resulted in an increase ($P<0.01$) in fat accretion rate compared to the pigs that remained on the rpST treatment. Bone accretion of pigs treated with rpST was higher ($P<0.05$) than that of pigs withdrawn from rpST treatment in the finishing period, and pigs of the other two groups during the overall experimental period (Table 4).

Plasma growth hormone

The average plasma growth hormone concentration was increased ($P<0.005$) by rpST injection in both the growing and finishing periods (Table 5). Withdrawal of rpST

injections resulted in reducing plasma growth hormone levels to the same as that in control pigs.

Bone measurements

Daily injection of rpST had no influence on the width of distal ulna physis in either the growing or finishing period, or on the other measurements of the last thoracic vertebrae or last ribs in the growing period (Table 5). However, the last thoracic vertebra and last rib from pigs that remained on rpST treatment to 100.3 kg liveweight contained lower concentrations of dry matter ($P<0.001$), lower ash on a moisture-free basis ($P<0.05$), lower dry matter weight ($P<0.01$) and lower ash weight ($P<0.001$) per cm^3 of bone than the same bones from control animals. The dry matter and ash content on a moisture-free basis and dry matter per cm^3 of vertebrae and ribs were intermediate for pigs withdrawn from rpST treatment. The ash weight per cm^3 of the bone from pigs withdrawn from rpST was lower than that of bones from the control and rpST treated pigs.

Visual evaluation of joint cartilage and physis

The visual scores for cartilage of humeral and femoral distal condyles and the distal ulna physis were not affected by the daily injection of rpST from 33.0 to 62.8 kg liveweight (Table 6). However, a higher visual score ($P<0.05$) for the distal humeral condyle was observed in finishing pigs treated with rpST compared to pigs from the control group.

Uronic acid and hydroxyproline concentrations in cartilage

Uronic acid and hydroxyproline concentrations of cartilage were not different between the distal humeral and femoral condyles, therefore, the values were pooled (Table 7). These chemical measurements were not affected by rpST injection during the growing period. However, both uronic acid and hydroxyproline concentrations were reduced ($P<0.05$ and $P<0.005$, respectively) by rpST injection of pigs from 33.0 to 100.3 kg. Withdrawal of rpST from pigs at 62.8 kg increased the uronic acid and hydroxyproline concentrations in the cartilage. The ratio of uronic acid to hydroxyproline was not different between treatments in pigs slaughtered at 62.8 or 100.3 kg liveweight.

D. DISCUSSION

Cartilage damage caused by exogenous growth hormone has been reported by many investigators (Machlin 1972; Grondalen and Vangen 1974; Lund-Larsen and Bakke 1975; Etherton et al. 1986; Evock et al. 1988). The present research demonstrates that the long term treatment of pigs with rpST increased cartilage lesions independently of its effect on growth rate. Pigs treated with rpST had a significantly higher lesion score in the distal humeral condyle (Table 6), which is the joint most frequently affected by OC, than that of control pigs even though the growth rate was not

significantly increased (Table 2). These results confirm our previous observation (Chapter III) that increased mechanical stress due to enhanced growth rate was not the only factor influencing the occurrence of OC in pigs.

The present experiment demonstrated that the uronic acid concentration of cartilage was reduced in pigs treated with rpST. In addition, cartilage hydroxyproline concentration in the joint cartilage was reduced by rpST injection (Table 7). Uronic acid concentration indicates cartilage glycosaminoglycan content and hydroxyproline is a component of collagen. Glycosaminoglycan and collagen contribute to cartilage its compressive and tensile strength, respectively (Poole 1986). These data suggested that long term treatment of pigs with rpST would reduce cartilage strength and may make it much more susceptible to mechanical stress. This speculation is in agreement with the observation of Harris (1950) that the shearing strength of growth cartilage was reduced after growth hormone injection in growing rats.

Growth hormone and insulin-like growth factor-I (IGF-I) are the major regulators of cartilage and bone growth. It is believed that growth hormone stimulates the differentiation of epiphyseal growth plate precursor cells and increases the responsiveness of chondrocytes to IGF-I, while IGF-I prepares cartilage for calcification by stimulating the maturation and hypertrophy of the chondrocytes (Isaksson et

al. 1987). Injection of rats with growth hormone and IGF-I (Russell and Spencer, 1985, Isgaard et al. 1986) increases cartilage thickness and stimulates chondrocyte growth. Pigs treated with rpST in the present experiment had significantly higher levels of plasma growth hormone, but no effect on the width of the distal ulna growth plate was noted (Table 5). It is possible that there is a difference between the long term and short term effects of growth hormone on chondrocyte maturation and function. A stimulatory effect on cartilage growth by growth hormone treatment was reported in rats after four (Russell and Spencer 1985) or eleven days (Isgaard et al. 1986) while our observations were made after 42 and 78 or more days of injections with rpST. Although there is no information regarding the long term effect of rpST on chondrocytes, our experiment demonstrated that the long term treatment with rpST resulted in a similar percentage reduction in the extracellular concentration of collagen and glycosaminoglycans, suggesting that the mechanism of rpST induced cartilage damage in pigs is through its effect on chondrocyte metabolism.

The increased percentage of carcass bone weight (Table 3) in rpST treated pigs can be ascribed to increased water content (Table 5) and this is in agreement with the observations of Caperna et al. (1989). Previous studies on the effects of rpST on bones from pigs revealed that the

femur, metacarpal and ribs appeared to be weaker and less mineralized (Goodband et al. 1993). Our experiment demonstrated similar effects of rpST on vertebrae and ribs (Table 5). The greater loss of bone minerals observed in the present experiment than that reported in previous studies (Goodband et al. 1993) may be explained by the fact that axial bones evaluated in our experiment are more sensitive to lack of mineralization than bones from the pig's extremities (Mahan and Fetter, 1982; Maxson and Mahan, 1986). Goodband et al. (1993) suggested that an increased cellular requirement for calcium and phosphorus combined with the lower feed intake usually observed with rpST treated pigs may explain the lower mineralization of bones of rpST treated pigs. Carter et al. (1991) reported that rpST treated pigs required higher dietary Ca and P levels to maximize bone strength and mineralization. However, an increase in dietary P content alone for rpST treated pigs may also promote bone mineralization and strength (Schricker 1990).

During the growing period in this experiment, rpST treated animals had 8.9% lower daily Ca and P intake and 9% higher daily bone weight gain (Table 4) than pigs of the control group. However, these differences had no significant influence on bone mineralization (Table 5), possibly due to increased rpST stimulated intestinal absorption (Heaney et al. 1972) and a greater retention of Ca and P (Verstegen et

al. 1990). In the finishing period, daily feed intake was reduced by 10.3% (Table 2) and daily bone weight gain was increased by 20.1% (Table 4) in pigs treated with rpST compared to the control animals. These differences resulted in a significantly reduced mineralization and density of bones from rpST treated pigs slaughtered at 100.3 kg (Table 5). Withdrawal of animals from rpST treatment during the finishing period improved the dry matter and ash content of the bones, presumably due to an increased mineral supply resulting from increased feed intake and reduced bone growth. However, ash density (wt/vol) of the bones was not increased to the levels observed in the control animals (Table 5). Reduced collagen content of cartilage, as indicated by lower hydroxyproline concentration, was observed in pigs treated with rpST (Table 7). It is possible that the collagen content of bones was also decreased which may have contributed to the lower mineralization of the bones in rpST treated pigs.

Our studies indicated that pigs treated with rpST may serve as a satisfactory model for the study of OC and that the cartilage damage observed may result from the effects of rpST on chondrocyte metabolism. Therefore, detailed research on the long term effect of exogenous growth hormone on the metabolism of chondrocytes and extracelluar matrix may increase our understanding of the pathogenesis of OC.

Table IV.1. Composition of experimental diets

	Dietary crude protein level				
	24	18	16	14	10
Ingredient (g/kg)					
Barley	400	577	630	686	791
Soybean meal (48% CP)	440	267	210	152	35
Sucrose	100	100	100	100	100
Tallow	15	20	25	27	38
Limestone	12	10	10	9	9
Dicalcium phosphate	19	12	11	10	11
Salt	4	4	4	4	4
Vitamin-mineral premix[z]	10	10	10	10	10
Lysine-HCl	-	-	-	2	2
Nutrient (as fed basis)[y]					
DE MJ/kg	13.72	13.68	13.71	13.69	13.69
Protein (%, N x 6.25)	24.0	18.02	16.0	13.98	9.98
Lysine (%)	1.47	1.01	0.95	0.85	0.61
Calcium	0.93	0.69	0.66	0.59	0.59
Phosphorus	0.83	0.63	0.59	0.55	0.53

[z]Vitamin mineral supplied the following per kg of diet: 5,000 IU vitamin. A, 500 IU vitamin. D, 40 mg vitamin. E, 30 µg vitamin. B_{12}, 12 mg riboflavin, 45 mg niacin, 25 mg pantothenic acid, 600 mg choline, 200 µg biotin, 200 µg folic acid, 150 mg iron, 20 mg manganese, 120 mg zinc, 125 mg coppor, 0.3 mg selenium.

[y]Determined values of four samples per diet. DE was calculated.

Table IV.2. Effect of rpST treatment on pig performance

	Hormonal treatment				
	Control	rpST	Withdrawal	SE[z]	Sig[y]
Growing period					
Number of pigs	32	32			
Initial weight (kg)	32.91	33.14		0.50	NS
Final weight (kg)	62.91	62.78		0.94	NS
Avg. daily weight gain (kg)	0.85	0.85		0.02	NS
Avg. daily feed intake (kg)	1.80[a]	1.64[b]		0.03	*
Feed/gain	2.12[a]	1.93[b]		0.03	*
Finishing period					
Number of pigs	24	12	11[x]		
Initial weight (kg)	62.19	63.05	60.27	1.25	NS
Final weight (kg)	100.68	99.13	100.74	0.52	NS
Avg. daily weight gain (kg)	0.99[a]	0.99[a]	0.88[b]	0.02	*
Avg. daily feed intake (kg)	2.43[a]	2.18[b]	2.59[a]	0.06	*
Feed/gain	2.45[b]	2.26[b]	2.94[a]	0.06	*
Overall					
Number of pigs	24	12	11[x]		
Avg. daily weight gain (kg)	0.92[a]	0.92[a]	0.83[b]	0.01	*
Avg. daily feed intake (kg)	2.09[a]	1.89[b]	2.12[a]	0.04	*
Feed/gain	2.25[b]	2.04[c]	2.55[a]	0.04	*

[z]Standard error.
[y]Significant level at *=P<0.05 or NS=not significantly different (P>0.05).
[x]One pig was removed from the experiment due to poor condition.
[abc]Values in the same row with different superscripts are different.

Table IV.3. Effect of rpST on carcass characteristics of pigs

	Hormonal treatment				
	Control	rpST	Withdrawal	SE[z]	Sig[y]
Growing Period					
Number of pigs	8	8			
Initial weight (kg)	34.18	34.23		0.62	NS
Final weight (kg)	64.38	65.90		0.39	NS
Carcass weight (kg)	42.14	41.71		0.40	NS
Fat (%)	26.39[a]	20.74[b]		1.04	*
Bone (%)	13.20	13.96		0.22	NS
Lean (%)	57.94[b]	62.83[a]		1.03	*
Growing and finishing period					
Number of pigs	24	12	11[x]		
Initial weight (kg)	32.14	33.22	32.43	0.65	NS
Final weight (kg)	101.09	101.82	100.83	0.59	NS
Carcass weight (kg)	65.91	63.76	65.65	0.49	NS
Fat (%)	28.79[a]	21.46[b]	27.76[a]	0.59	*
Bone (%)	12.03[b]	13.34[a]	12.34[b]	0.14	*
Lean (%)	56.98[b]	62.85[a]	57.78[b]	0.51	*

[z]Standard error.
[y]Significant level at *=P<0.05 or NS=not significantly different (P>0.05).
[x]One pig was removed from the experiment due to poor condition.
[ab]Values in the same row with different superscripts are different.

Table IV.4. Effect of rpST on the rate of tissue accretion in pigs

	Hormonal treatment				
	Control	rpST	Withdrawal	SE[z]	Sig[y]
Growing Period					
Number of pigs	8	8			
Lean (g/d)	335.73	378.29		12.69	NS
Fat (g/d)	175.98[a]	117.17[b]		11.31	**
Bone (g/d)	67.45	73.52		2.07	NS
Finishing Period					
Number of pigs	24	12	11[x]		
Lean (g/d)	370.24[b]	445.97[a]	327.09[b]	18.57	**
Fat (g/d)	268.70[a]	119.23[b]	222.81[a]	15.54	**
Bone (g/d)	70.00[ab]	84.10[a]	62.96[b]	3.56	*
Overall					
Number of pigs	24	12	11[x]		
Lean (g/d)	352.71[b]	389.84[a]	341.32[b]	8.13	**
Fat (g/d)	197.98[a]	118.52[b]	180.32[a]	6.52	**
Bone (g/d)	68.42[b]	75.50[a]	66.35[b]	1.78	*

[z]Standard error.
[y]Significant level at *=P<0.05, **=P<0.01 or NS=not significantly different (P>0.05).
[x]One pig was removed from the experiment due to poor condition.
[ab]Values in the same row with different superscripts are different.

Table IV.5. Effect of rpST on plasma growth hormone concentration and bone measurements

	Growing period				Growing and finishing period				
	Control	rpST	SE[z]	Sig[y]	Control	rpST	Withdrawal	SE[z]	Sig[y]
Number of samples	32	32			24	12	11[x]		
Growth Hormone (ng/mL)	2.96[b]	5.37[a]	0.31	***	1.81[b]	3.44[a]	1.70[b]	0.15	***
Width of distal ulna physis (mm)	0.75	0.93	0.10	NS	0.50	0.60	0.55	0.03	NS
Last thoracic vertebra									
Dry matter (%)	57.51	58.61	1.29	NS	63.34[a]	59.32[b]	62.35[a]	0.36	****
Ash weight (% dry matter)	49.81	51.64	0.92	NS	48.63[a]	46.21[b]	47.53[ab]	0.37	*
Dry matter density (g/mL)	0.70	0.83	0.04	NS	0.98[a]	0.77[b]	0.86[ab]	0.03	**
Ash density (g/mL)	0.35	0.43	0.03	NS	0.50[a]	0.37[b]	0.41[b]	0.01	****
Last rib									
Dry matter (%)	61.22	59.98	0.74	NS	65.74[a]	62.45[a]	64.36[ab]	0.43	**
Ash weight (% dry matter)	45.84	45.71	0.89	NS	47.34[a]	45.71[b]	45.93[ab]	0.31	*
Dry matter density (g/mL)	1.01	1.11	0.07	NS	1.16[a]	0.95[b]	1.06[ab]	0.03	**
Ash density (g/mL)	0.46	0.51	0.03	NS	0.54[a]	0.42[c]	0.49[b]	0.01	****

[z]Standard error.
[y]Significant level at *=P<0.05, **=P<0.01, ***=P<0.005, ****=P<0.001 or NS=not significantly different (P>0.05).
[x]One pig was removed from the experiment due to poor condition.
[ab]Values in the same row within a same growth period with different superscripts are different.

Table IV.6. Effect of rpST on visual score of distal humeral and femoral condyles and distal ulna physis[z]

	Growing period				Growing and finishing period				
	Control	rpST	SE[y]	Sig.[x]	Control	rpST	Withdrawal	SE[y]	Sig[x]
Number of sample	32	32			24	12	11[w]		
Humeral condyle	2.0	2.3	0.14	NS	2.1[a]	2.6[b]	2.2[ab]	0.11	*
Femoral Condyle	2.0	2.7	0.14	NS	1.9	2.3	2.3	0.19	NS
Distal ulna physis	2.0	2.3	0.54	NS	2.2	2.5	2.5	0.24	NS

[z]See Materials and Methods for scoring system.
[y]Standard error.
[x]Significant level at *=P<0.05 or NS=not significantly different (P>0.05).
[w]One pig was removed from the experiment due to poor condition.
[ab]Values in the same row and within the same growth period with different superscripts are different.

Table IV.7. Effect of rpST on uronic acid and hydroxyproline concentrations in cartilage from the weight bearing area of distal humeral and femoral condyles[z]

	Growing period				Growing and finishing period				
	Control	rpST	SE[y]	Sig[x]	Control	rpST	Withdrawal	SE[y]	Sig[x]
Number of sample	32	32			24	12	11[w]		
Uronic acid	7.1	8.2	0.47	NS	7.0[a]	6.2[b]	6.6[ab]	0.12	*
Hydroxyproline	6.7	7.0	0.10	NS	7.5[a]	7.1[b]	7.2[ab]	0.06	***
Uronic acid/hydroxyproline	1.07	1.21	0.08	NS	0.94	0.92	0.93	0.02	NS

[z]Percentage of dry cartilage weight.
[y]Standard error.
[x]Significant level at *=P<0.05, ***=P<0.005 or NS=not significantly different (P>0.05).
[w]One pig was removed from the experiment due to poor condition.
[ab]Values in the same row and within a same growth period with different superscripts are different.

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V. EFFECTS OF LONG TERM TREATMENT WITH RECOMBINANT PORCINE SOMATOTROPIN (rpST) ON THE CHEMICAL COMPOSITION OF DIFFERENT LAYERS OF JOINT CARTILAGE IN PIGS

A. INTRODUCTION

The administration of recombinant porcine somatotropin (rpST) has been shown to induce a high incidence and severity of osteochondrosis (OC) in growing and finishing pigs (Evock et al. 1988). This effect has more recently been demonstrated to be associated with reduced collagen and glycosaminoglycan concentrations in the weight-bearing area of joint cartilage (Chapter III and IV). It has also been reported that the glycosaminoglycan content, as indicated by uronic acid concentration (Nakano et al. 1985), is decreased in osteochondrotic cartilage tissue (Nakano et al. 1979a, b). However, the change in keratan sulfate, the second most abundant of the glycosaminoglycans, in OC affected cartilage is not reflected in the concentration of uronic acid because keratan sulfate does not contain uronic acid. Chondroitin sulfate and keratan sulfate attach to the core protein at different domains, though the significance of their arrangement in proteoglycan is not known. There is no available data on the effects of rpST treatment of pigs on the keratan sulfate content of joint cartilage.

Joint cartilage can be histologically divided into articular cartilage and epiphyseal cartilage, and the latter

can be subdivided into resting, proliferating, hypertrophic and calcifying zones, representing the morphological changes of chondrocytes (Carlson et al. 1985). Researchers recently proposed (Nakano and Aherne, in press) that chondrocytes in the proliferating zone may be more susceptible to mechanical stress, and this susceptibility may be associated with the disorganization of collagen ultrastructure and reduced cartilage proteoglycan content. Such changes may result in reduced tensile strength and compressive strength of cartilage and hence to an increased incidence of osteochondrosis (Freemen et al. 1972). However, there is lack of information to support this hypothesis.

In the present experiment, we studied joint cartilage from pigs treated with or without rpST to determine the effect of rpST on concentrations of hydroxyproline, uronic acid and total sulfated glycosaminoglycans in different zones of joint cartilage and to study their relationship to OC.

The experiment was reviewed and approved by the University of Alberta Animal Care Committee to ensure adherence to Canadian Council of Animal Care guidelines.

B. MATERIALS AND METHODS

Seventy-two pigs (PIC, Camborough X Canabrid, equal number of gilts and barrows) at an average liveweight of 56.1±6.4 kg were allotted to 12 pens on the basis of sex and

weight. The pens were approximately 3.0 X 1.5 m in size, with half slatted concrete floors. Pigs were fed ad libitum a 17.4% crude protein diet (Table 1) and had free access to water. Two treatments, daily injection of rpST (Pitman-Moore, Terre Haute, IN) at 3 mg per pig per day and daily saline injection (Control), were randomly assigned to pens. Feed intake and weight gain were recorded weekly. Pigs were slaughtered when the average live weight per pen reached 106.5±6.3 kg.

Distal humerus and proximal radial-ulna with intact elbow joint were collected at the time of slaughter. Cartilage soundness of the humeral condyles was visually evaluated following the subjective scoring system introduced by Brennan and Aherne (1986). A transverse strip of cartilage (approximately 1 x 3 cm, width x length) from the weight bearing area of each humeral condyle was collected. Part of the sample containing articular cartilage, subarticular cartilage and ossification layer was fixed in 4% buffered formalin with 0.5% cetylpyridinium chloride (Williams and Jackson 1956), dehydrated and embedded in paraffin by routine procedures (Drury and Wallington 1967). Seven micron thick sections were cut vertically to the articular surface and stained with haematoxylin and eosin (Drury and Wallington 1967) for histological study. The remaining cartilage was divided, using surgical blade, into top, middle and bottom layers which corresponded to the

articular cartilage and resting zone, proliferating zone, and hypertrophic and calcifying zones, respectively (Fig. 1). The cartilage samples were acetone dried and digested with papain (Nakano et al. 1979a). A portion of the papain digesta was assayed for hydroxyproline (Stegeman and Stalder 1967) and sulphated glycosaminoglycan content (Farndale et al. 1982). The remaining portion of the digesta was deproteinized by adding trichloroacetic acid to a final concentration of 7%. Following removal of protein by centrifugation, the supernatant was dialysed exhaustively against water. The dialysate was used for determination of total uronic acid (Kosakai and Yoshizawa 1979).

Data were analyzed using General Linear Model procedures of SAS (1985). Factors included in the model were sexes (2) and treatments (2) for analysis of performance and cartilage scoring data, and sexes (2), treatments (2) and cartilage layers (3) for analysis of cartilage chemical measurements. Preliminary analysis indicated that there was no interactive effect of sex with other factors, therefore, sex was removed from the model.

C. RESULTS

Daily injection of rpST increased ($P<0.04$) daily weight gain and marginally reduced ($P<0.06$) the feed/gain ratio without affecting ($P>0.94$) daily feed intake. Cartilage lesion score was marginally increased ($P<0.07$) in pigs

injected daily with rpST compared with pigs of the control group (Table 2).

Histological examination of cartilage demonstrated that rpST treatment resulted in OC lesions in the hypertrophic zone (Fig. 1) characterized by an absence of chondrocytes. Clusters, each containing several hypertrophic chondrocytes, surrounded these areas. These lesions were seen in all zones of the growing cartilage, including the area of cartilage retention in the subchondral bone.

In the control animals, the top layer of joint cartilage contained less sulfated GAGs than the middle and bottom layers. Uronic acid concentration was not different between layers, but hydroxyproline content was considerably lower in the bottom layer, which resulted in a significantly higher ratio of uronic acid to hydroxyproline (Table 3).

Injection of pigs with rpST did not affect the chemical composition of the top layer but significantly reduced concentrations of sulfated GAGs, uronic acid and hydroxyproline in the middle and bottom layers of the joint cartilage, compared with the control pigs (Table 3). However, the ratios of uronic acid to hydroxyproline in each layer of joint cartilage were not different between treatment groups (Table 3).

D. DISCUSSION

There is limited information regarding the chemical

composition of different layers of joint cartilage. Using immunohistochemical methods, Horton and Machado (1988) observed that collagen type II is evenly distributed throughout the growth plate of physis and that the calcification zone is rich in proteoglycans. Data from the current experiment shows that hydroxyproline concentration is higher in the middle layer than in the bottom layer and proteoglycans, as measured by uronic acid and sulfated GAGs, are richer in the middle and bottom layers. Joint cartilage is located at the end of bones and sustains weight loading, wearing and friction (Mow et al. 1992) while the growth plate of the physis is located between the epiphyseal and metaphyseal bones and may be less important as a weight bearing tissue than joint cartilage (Nakano and Aherne in press). The variation in their function may require differences in strength and hence the difference in chemical composition.

Although failure of ossification in subchondral bone is a typical symptom of OC, early lesions occur in the subarticular growth cartilage (Ekman et al. 1990). It has been suggested that the subarticular cartilage is a biomechanically weak tissue and is susceptible to mechanical stress which in turn leads to increased enzymatic degeneration of cartilage matrix and loss of cartilage collagen and proteoglycan (Nakano and Aherne in press). In agreement with their hypothesis, our experiment demonstrated

that the high incidence of OC observed in rpST treated pigs was associated with reduced hydroxyproline and glycosaminoglycan concentration in the subarticular cartilage, particularly in the differentiating, maturation and hypertrophic zones.

Reduction of hydroxyproline concentration in OC cartilage was also reported by other investigators (Nakano et al. 1979a). Although the present experiment did not determine what was the cause of the low content of hydroxyproline in subarticular cartilage, it can be speculated that cartilage tensile strength was reduced by rpST treatment.

Difference in composition of macromolecules in different layers of joint cartilage could facilitate chondrocyte growth, cartilage functioning and cartilage calcification (Ekman et al. 1990). An abnormal macromelecular composition in the cartilage could lead to a failure of endochondral ossification, followed by a typical OC lesion. It has been suggested that changes in the composition, structure and organization of proteoglycans may be the essential condition for calcification to occur (Poole et al. 1083). Therefore, the failure of chondrocytes to modify the composition, structure and organization of proteoglycan in the extracellular matrix can be considered a primary cause of OC. Our results demonstrated a different chemical composition in joint cartilage from pigs treated

with rpST, indicating an abnormal chondrocyte activity in the modification of the extracellular matrix.

It is known that growth hormone and IGF-I are major regulators of cartilage growth (Isaksson et al. 1987). Growth hormone directly stimulates chondrocyte differentiation and local production of IGF-I, while IGF-I increases cartilage growth in thickness by regulating chondrocyte maturation and hypertrophy (Isaksson et al. 1987). Chondrocytes modify the chemical composition of the extracellular matrix by synthesizing and degrading macromolecules including collagen and GAG (Mow 1992). Failure of a local effect of IGF-I on chondrocytes may be the cause of the reduced collagen and GAGs observed in the cartilage of rpST treated pigs. This speculation is supported by the observation that the thickness of the distal ulna physis was not increased after long-term treatment with rpST in pigs (Chapter VI). Furthermore, reduction of hydroxyproline, uronic acid and sulfated GAGs (Table 3) and early lesions of chondronecrosis, which develop into retained cartilage in subchondral bone (Ekman et al. 1990), are observed in zones where chondrocyte growth and metabolism is regulated by IGF-I.

There is no available information regarding the mechanism by which the long-term treatment with rpST affects IGF-I production. It is suggested that somatostatin levels in plasma of rpST treated pigs is increased because

somatostatin is a major hormone controlling the baseline of plasma growth hormone (Tannenbaum and Ling 1984). Somatostatin directly inhibits secretion and activity of IGF-I in many cell types, including chondrocytes (Lamberts 1988). Infusion of somatostatin to mice resulted in an inhibition of longitudinal bone growth (Marie et al. 1988), presumably due to somatostatin blocked IGF-I secretion in cartilage (Mackie et al. 1990). It may be speculated that rpST treatment caused reduction of proteoglycan and collagen contents in layers where IGF-I regulates chondrocyte growth and metabolism through an inhibitory effect of increased somatostatin concentration. Therefore, study of the relationship between plasma somatostatin concentration and IGF-I production and secretion in maturation and hypertrophic zones of joint cartilage may reveal a possible mechanism by which rpST treatment causes a high incidence of OC in pigs.

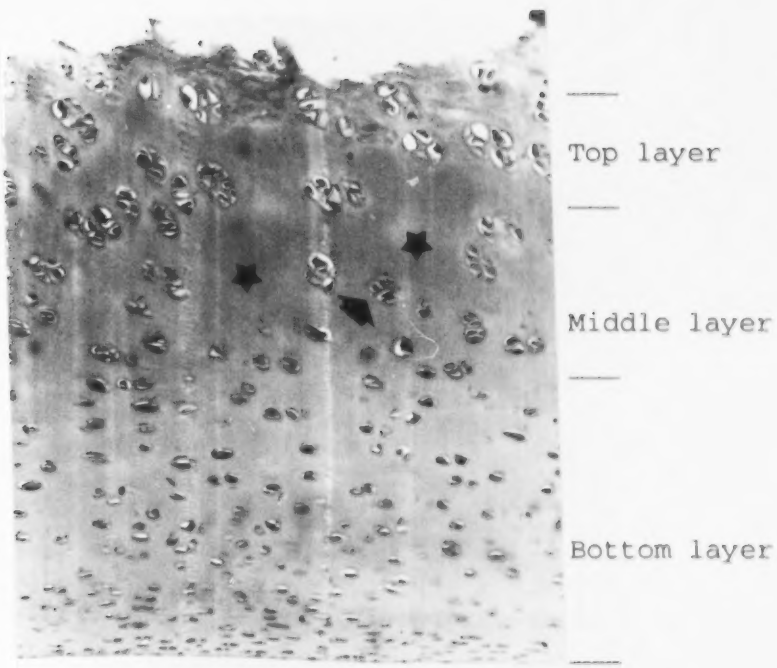

Figure V.1. Histological sections of articular-epiphyseal cartilage of distal humeral condyle from rpST treated pigs. Section was made vertically to the articular cartilage surface and shows top, middle and bottom layers. Cartilage was stained with hematoxylin and eosin. Cartilage from rpST treated pigs. Areas of degenerated cartilage ( ) were present in the hypertrophic zones. The degenerated cartilage is surrounded by clustered chondrocytes (arrow).

Table V.1. Composition of experimental diet

Ingredient (%)	
Wheat	33.9
Barley	46.0
Soybean meal (47.5% CP)	16.0
Limestone	1.0
Dicalcium phosphate	0.3
Salt	0.3
Vitamin-mineral premix[z]	2.5
Nutrient[y]	
DE (MJ/kg)	13.17
Crude protein (N x 6.25, %)	17.4
Lysine (%)	0.9
Calcium (%)	0.72
Phosphorus (%)	0.58

[z]Provided 1.5 times the NRC (1988) recommended levels.
[y]As fed basis. DE and lysine are calculated values.

Table V.2. Effect of rpST on growth performance and joint cartilage soundness of pigs

	Control	rpST	SE[z]	Probability
Number of pigs	36	36		
Number of pens	6	6		
Average initial weight (kg)	56.16	55.87	2.90	0.78
Average final weight (kg)	106.25	106.79	0.75	0.82
Avg. daily weight gain (kg)	0.84	0.94	0.02	0.04
Avg. daily feed intake (kg)	2.89	2.82	0.06	0.62
Feed/gain	3.43	3.04	0.14	0.06
Cartilage visual score[y]	1.65	1.98	0.14	0.07

[z]Standard error.
[y]Visual evaluation of humeral condyles: 1=normal and 5=severe lesions.

Table V.3. Chemical analysis of different layers of joint cartilage from weight bearing area of the distal humeral condyle

	Control			rpST			
	Top	Middle	Bottom	Top	Middle	Bottom	SE[z]
Number of condyles	36	36	36	36	36	36	
Sulfated glycosaminoglycans (% Dry matter)	26.0[bc]	29.7[a]	29.3[a]	25.3[c]	27.1[b]	27.1[b]	0.63
Uronic acid (% dry matter)	6.5[ab]	6.5[a]	6.9[a]	6.1[bc]	6.1[bc]	5.9[c]	0.16
Hydroxyproline (% dry matter)	6.5[ab]	6.7[a]	5.7[b]	6.3[bc]	6.2[c]	4.8[d]	0.10
Uronic acid/hydroxyproline	0.99[b]	0.97[b]	1.21[a]	0.96[b]	0.97[b]	1.25[a]	0.03

[z]Standard error.
[abcd]Values within the same row with the same superscript were not significantly different (P>0.05).

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VI. GENERAL DISCUSSION

The present research is the first to differentiate the effects of mechanical stress due to enhanced growth rate from the effects of exogenous growth hormone (GH) on the occurrence of osteochondrosis (OC) in pigs. Our hypothesis was that an increase of growth rate or carcass lean in growing-finishing pigs may not cause a high incidence or severity of OC unless accompanied by an increase in plasma levels of GH. To achieve a satisfactory experimental model, the ß-adrenergic agonist ractopamine was added to diets of growing-finishing pigs. This growth promoter acts in a manner similar to that of naturally occurring catecholamines and elicits its biological responses by direct binding to ß-adrenergic receptors on its target cells (Etherton and Smith 1991). Although there is no information regarding the effect of ß-adrenergic agonist on GH secretion, hypophysectomized rats have been shown to respond to the stimulating effect of ß-adrenergic agonist (Thiel et al. 1987), indicating that ß-adrenergic agonist enhanced growth is independent of the effects of GH. Lesion scores and uronic acid concentrations of joint cartilage were not affected by increased growth rate obtained from feeding ractopamine, which suggests that mechanical stress is not the only factor, or perhaps even the most important factor causing OC in growing-finishing pigs. Our observations are in agreement with previous reports which showed that decreasing the growth rate of pigs

by manipulating nutrient intake had no significant effect on the occurrence of OC (Nakano et al. 1979; Kornegay et al. 1981).

It seems our results conflict with those in which local overloading was used to provide mechanical stress on joint cartilage. Lesions associated with loss of proteoglycans in the weight bearing area of the medial humeral condyle were observed in pigs carrying loads of 15% of their liveweight from 20 to 42 kg (Brennan et al., 1987). Short periods (two to four weeks) of overloading of the elbow joint in growing pigs increased osteochondrotic lesions in the distal epiphyseal plate of the ulna (Walker et al. 1966; Grondalen and Grondalen 1974). However, the overloading was confounded with reduced exercise because overloaded pigs spent less time standing and walking than control animals (Brennan et al. 1987). Studies suggested that exercise is essential for normal growth of cartilage and that lack of exercise leads to retarded cartilage growth (Steinberg and Trueta 1981). Therefore, overloading experiments may not provide a satisfactory model to support the hypothesis that mechanical stress from enhanced growth rate is an important factor in causing a high incidence of OC in pigs.

A positive relationship between enhanced growth rate and the high incidence of OC has been observed in pigs genetically selected for fast growth rate and lean carcass (Grondalen and Vagen 1974; Geodegebuure et al. 1980). Pigs

with higher cartilage lesion scores grew quickly and were leaner at slaughter than pigs with low lesion scores (Lundeheim, personal communication). Mechanical stress on joint cartilage caused by growth rate and high levels of plasma GH were confounded as possible causes of OC in these experiments.

The second experiment in this thesis successively differentiated between the effect of high plasma GH and enhanced growth rate with its concomitant increase in mechanical stress on joint cartilage. Our results clearly demonstrate that high levels of GH may be a predisposing factor causing a high incidence of OC in fast growing pigs, because regardless of growth rate, an increase in cartilage lesion scores and a decrease in uronic acid concentrations occurred in all pigs treated with rpST. It may be speculated that genetically selected fast growing pigs with high plasma GH levels are more prone to a high incidence of OC than pigs with lower growth rates. Restricted feeding or providing diets with low nutrient density may not influence plasma GH levels (Campbell et al. 1990), which may partially explain why reduced growth rate by restricted feeding had no influence on the incidence of OC in pigs (Fell et al. 1970; Grondalen 1974; Nakano et al. 1979; Arther et al. 1983) .

Administration of rpST in pigs increases growth rate and this effect is dependent on the method of rpST delivery. Daily injection of rpST gives large peaks in the circulating

concentration of GH, which simulates the natural secretion pattern and is required to activate the physiological processes necessary for growth enhancement. A positive relationship between frequency of rpST injection and growth rate of pigs has been observed (Evock and Steele 1991). Constant infusion of GH in rodents did not elicit as stimulatory effect as pulsative infusion (Norstedt and Palmiter, 1984). It is not surprising therefore, rpST administrated by slow release implant, a method of continuous administration, gave a different response in growth enhancement (Chapter III), even though both daily injection and implant delivered the same amount of rpST daily.

It is generally believed that GH has a positive effect on cartilage growth. Growth hormone stimulates chondrocyte production and differentiation. It also stimulates the secretion of IGF-I by chondrocytes which in turn promotes chondrocyte maturation and hypertrophy. However, these observations were obtained with hypophysectomized animals treated with GH for a short period (see review by Isaksson et al. 1987). Injecting GH into hypophysectomized animals restores this hormone to a normal plasma level and the observed improvement in cartilage growth is more of a restorative effect. Administration of GH to animals with intact hypothalami increased its basal level in plasma (Chapter IV) which may have stimulated the endogenous

secretion of somatostatin. Somatostatin is one of the major regulators controlling the baseline level of GH in the animal body (Tannenbaum and Ling 1984). Long term treatment of intact animals with growth hormone, therefore, may be accompanied by high levels of somatostatin. It has been observed that infusion of somatostatin into the mouse result in a inhibition of longitudinal bone growth (Marie et al. 1988). Because somatostatin directly inhibits the secretory activities of cells (Reichlin 1983a, b; Lamberts 1988), it was speculated that somatostatin blocked secretion of IGF-I in chondrocytes (Mackie et al. 1990). Our experiment provided further evidence that cartilage concentrations of glycosaminoglycan and hydroxyproline were decreased in rpST treated pigs (Chapter IV) and this reduction of macromolecules was significant in the maturation, hypertrophic and calcifying zones of joint cartilage (Chapter V). Because chondrocyte metabolism in these areas is controlled by IGF-I, it may be speculated that IGF-I production and secretion are inhibited by rpST induced high levels of somatostatin.

Synthesis and degradation of the extracellular matrix are two spontaneous processes carried out by chondrocytes in cartilage (Mow and Ratcliffe 1992). The major macromolecules in joint cartilage are collagen and proteoglycan which provide cartilage with tensile strength and compressive strength, respectively. There is a hypothesis in the

literature which states that fatigue of collagen (Freeman 1972) and depletion of proteoglycan (Nakano and Aherne, in press) may be the primary factors leading to cartilage damage. However, information regarding the relationship between these macromelecular concentrations and the occurrence of OC in pigs is limited. The present research provided some evidence that the weight bearing areas of joint cartilage from pigs with a high incidence of OC contains a lower concentrations of collagen and proteoglycan.

The cartilage content of GAGs is usually estimated by measuring the uronic acid concentration because it is the component of chondroitin sulfate which composes 90% of total GAGs in cartilage (Nakano et al. 1987). However, quantitative changes of keratan sulfate concentrations are not measured by uronic acid because keratan sulfate contains no glucuronic acid. Keratan sulfate is covalently attached to core protein at a domain close to the link proteins (Barry 1990), the concentration change of keratan sulfate may not only affect cartilage compressive strength, but also the stability of aggregation between proteoglycan and hyaluronic acid. Therefore, determination of total sulfated GAGs in the joint cartilage may more precisely measure the GAG concentration and hence the compressive strength of joint cartilage. Our experiment (Chapter V) demonstrated that the concentration of total sulfated GAGs was similarly

affected by rpST treatment as was uronic acid-containing GAGs, indicating that long term treatment with rpST causes a general decrease of GAG concentration in joint cartilage. This observation may further support our speculation that chondrocyte metabolism is depressed by long term treatment of rpST in pigs.

Although long term treatment of pigs with rpST resulted in a high incidence of OC, none of the affected animals showed obvious lameness. Therefore, rpST treatment of growing-finishing pigs may not be an animal welfare concern. However, animals selected for breeding should not be treated with rpST because it may lead to OC and culling due to lameness in later life.

Based on our results, it may be concluded that mechanical stress due to enhanced growth rate, or high lean tissue deposition rate in fast growing pigs is not a primary factor causing high incidence of OC. However, increased plasma growth hormone levels through administration of exogenous growth hormone for a long period may result in a high incidence and increased severity of OC, probably by reducing proteoglycan and collagen content in weight-bearing area of joint cartilage. This effect is independent of mechanical stress due to enhanced growth rate.

The present research used cartilage from weight bearing area of joint cartilage demonstrated an effect of long term treatment of rpST on cartilage chemical composition.

However, it is not know if the same effect on non-weight bearing area of joint cartilage occurs. Further research should be conducted to compare the effect of long term treatment of rpST on joint cartilage from both cartilage locations.

To determine if high levels of somatostatin are associated with inhibition of chondrocyte growth and the occurrence of OC, experiments using somatostatin antibody should be conducted. The possible inhibitory effect of somatostatin on the activities of GH and IGF-I may be eliminated in animals treated with somatostatin antibody. A study of chondrocyte metabolism and the effects on extracellular matrix under such conditions may reveal the influence of rpST on chondrocytes and possibly the etiological mechanism of rpST induced OC in pigs.

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